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HARVESTING OF ALGAE BY FROTH FLOTATION

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RESOURCES RESEARCH INC.
A SUBSIDIARY OF HAZLETON LABORATORIES, INC.

Final Report

HARVESTING OF ALGAE BY FROTH FLOTATION

Public Health Service
Contract No. PH 86-63-75

RESOURCES RESEARCH, INC.
A Subsidiary of HAZLETON LABORATORIES, INC.

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HARVESTING OF ALGAE BY FROTH FLOTATION

SUMMARY

Efforts made during the current year have been concerned mainly with the determination and quantification of the factors that affect the economics of the froth flotation harvesting process. Three criteria can be used in evaluating the process: (a) percentage clarification of feed suspensions; (b) absolute amounts of harvest per unit of raw materials required; and (c) absolute amounts of harvest obtained per unit time. It was suggested that (a) above has little value, since percentage clarification is related only to the density or solids content of a given feed suspension. It was further suggested that, for both unialgal cultures and lagooned sewage, froth flotation processing can be evaluated best in terms of actual amounts of material removed from or remaining in feed suspensions.

Investigations were conducted to determine the optimum aeration rate and duration of aeration for froth flotation harvesting of unialgal cultures. For the various conditions tested, the amount of harvest was greatest when feed suspensions acidified to pH 3.0 were harvested at an aeration rate of 40 cc/min. for three min.

Harvest trials were conducted on one to five day old cultures ranging in initial pcv (packed cell volume) from 0.0005 to 0.0041 ml/ml to study harvesting economics. Harvest efficiency declined with age for all inoculum levels, and the efficiency of harvest was inversely proportional to culture density. However, the absolute amounts of harvest were not proportional to the efficiency of harvesting: dilute suspensions harvested at high efficiencies thus yielded less harvest mass than did dense cultures harvested at considerably lower efficiencies. Moreover, cultures initiated at a pcv of 0.0041 ml/ml yielded much more harvest per unit of air and acid expended than did cultures of the other inoculum levels. It was found that the maximum amount of harvest was obtained when relatively dense starting cultures (e.g. pcv 0.0041 ml/ml) were grown for about two days, and harvested. A minimum density below which cultures could not be economically harvested was not observed -- the most dilute cultures tested yielded about the same amount of total harvest as cultures having twice the cell concentration.

Studies on the growth and viability of Chlorella pyrenoidosa indicated that the organism is capable of growth on a variety of media such as fresh urea or KNO_3 medium, supplemented or unsupplemented harvest liquor, and diluted harvest liquor. Moreover, it was found that algal cells remained viable after prolonged exposure to the low pH conditions of flotation harvesting, and that cultures grew well after several growth-harvest cycles.

The cost of harvesting algae by froth flotation was re-evaluated. The cost of harvesting and drying one ton of dry algae by flotation harvesting relatively dense cultures of algae would be approximately $47.00. Based on an estimated value of $80 to $100/ton of dry algae, the process borders on economic feasibility.

Froth flotation harvesting of algae in sewage lagoons was studied. It was found that, by aerating sewage lagoon samples at ambient pH, "detergent" foam resulted and could be removed, but the algal harvest was negligible. However, after acid pH adjustment, foaming phenomena typical of unialgal cultures could be demonstrated and the algae could be harvested. Acid pH adjustment and froth flotation greatly reduced total bacterial populations, and completely eliminated coliform organisms in the clarified waste. Froth flotation, therefore, might be studied as an additional step in treatment of lagoon sewage and, perhaps, in conventional sewage treatment.

Efficiency of removal of lagoon suspended solids per unit of air expended was greatest when feed suspensions were aerated at a rate of 40 cc/min/450 ml suspension for 20 sec. Virtually complete removal of solids was achieved after 1200 sec of froth flotation, regardless of aeration rate. If complete clarification of lagooned sewage is desired, it is much less expensive, in terms of cost of air, to clarify suspensions at a low aeration rate (40 cc/min) than at higher aeration rates (90 or 155 cc/min). Likewise, if partial removal of algae to maintain populations in the log phase of growth is desired, it is more economical to harvest lagooned sewage at the 40 cc/min rate. Solids removals from alkaline adjusted feed suspensions and from acidified feed suspensions were equal. However, in terms of cost of acid and base for pH adjustment, it is much cheaper to harvest acidified suspensions than alkaline ones: $28.00 per million gallons adjusted to pH 3.0 versus about $278 per million gallons adjusted to pH 12.0.

It was found that the Jackson candle turbidimetric method was satisfactory for purposes of measuring densities of unialgal cultures and the suspended solids in lagooned sewage.

INTRODUCTION

A continuing effort was made during the current year to examine froth flotation as an economical means for harvesting relatively dilute suspensions of algae.

Levin et al. (1) reported that laboratory-grown cultures of algae frothed without the addition of flotants, which indicated that a frothing agent was produced by the cultures themselves. A high-temperature strain of Chlorella pyrenoidosa was selected for use in these studies because its adaptability to the conditions of laboratory culture and its relatively high reproductive rate make it desirable for mass culturing as a food source or for gas exchange in closed systems. They also described a modified method of froth flotation in which virtually complete harvesting of cells was obtained. Following acid pH adjustment of the feed culture, it was placed in a glass cylinder equipped with a porous diffusion plate at the bottom. Air was forced through the plate, creating bubbles in the liquid above. A stable foam, highly concentrated in algae, formed at the liquid surface and rose up the column; the foam was collected at a discharge orifice. The feed solution was virtually clarified by this process. Hydrochloric acid was used to lower the pH of the harvest feed cultures and sodium hydroxide for subsequent neutralization.

If the harvest liquor (culture supernatant after cell removal) can be recycled, and if it will again support good algal growth with or without a degree of nutrient supplementation, batch-type culturing and harvesting can be avoided. If the harvest liquor is to be recycled for continuous algal culturing, the medium must be neutralized. The harvested algal concentrate is acidic, and the dried product would be prohibitively acidic for use as a foodstuff unless the harvest were neutralized. Therefore, when mass culturing and harvesting operations are considered, the costs of air, acid, and base become important.

Modified froth flotation harvesting is less complicated than previously described (2) harvesting methods and the froth-harvesting apparatus described above is attractive for integration with a mass culturing system. In 1962, design and fabrication of a mass culture apparatus adaptable to continuous culturing and continuous flotation harvesting was accomplished. A detailed

description of the mass culture apparatus appears in a previous report (3). Studies utilizing this system have been concerned mainly with investigation of conditions amenable to good growth in cultures of volumes of 100 l or more.

It was of interest to determine if the algae, and, perhaps, waste material present in sewage oxidation lagoon effluent could be removed by flotation harvesting. Factors associated with efficient and economical clearing of sewage were investigated.

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INVESTIGATIONS ON THE GROWING AND HARVESTING OF ALGAE

Small-scale trials usually provide reliable guidelines for the design and operation of full-scale systems. The growth and harvesting studies discussed in this section were made on algal cultures grown in one-gallon jugs. Investigations described here were designed to give dimension to the following parameters: (1) growth of algae exposed to several growth-harvest cycles; (2) growth of algae on media ranging in nutrient content from that of fresh medium to that of dilute, recycled harvest supernatant; (3) tolerance of algae to prolonged exposure to low pH; and, (4) minimum and maximum culture densities that can be harvested with an acceptable expenditure of raw materials such as air, acid, and base.

Based on data obtained in the above investigations, the costs of flotation harvesting to produce large quantities of dry algal product were re-evaluated.

Viability and Growth

In the operation of a continuous culturing and harvesting system, it might be operationally, as well as economically, desirable to harvest only a portion of the algae and to recycle the remaining culture for further growth. Successful operation of such a system requires that the algae remain viable despite exposure to the low pH condition in the harvester. Investigations were directed toward determination of viability of cells repeatedly exposed to harvesting conditions, and their subsequent growth in fresh media, supplemented, unsupplemented, and diluted harvest liquor.

The basal medium used for culturing the algae was composed of the following nutrients made up to one liter in deionized-distilled water:

	Compound	Quantity (mg)
	$MgSO_4 \cdot 7H_2O$	1000
	KH_2PO_4	250
Sequestrene	$Na \cdot Fe$	35
"	$Na_2 \cdot Mn$	3
"	$Na_2 \cdot Cu$	1
"	$Na_2 \cdot Zn$	1
"	$Na_2 \cdot Co$	1

When KNO_3 was used in the medium, it was added in a concentration of 3.4 g/l, and when urea was used as the nitrogen source instead of KNO_3 it was added in a concentration of 1.0 g/l so that nitrogen concentrations would be equal in the two media.

Viability of Recycled Cells in Various Media

The viability of cells cultured under conditions of repeated recycling was determined in several media. One was a mixture of urea harvest liquor, deionized-distilled water and urea medium. Another was a mixture of KNO_3 harvest liquor, deionized-distilled water and KNO_3 medium. Cells grown in these media were compared to fresh cells grown in fresh urea and KNO_3 media.

Two 700 ml aliquots of medium, one containing urea as a nitrogen source, the other containing KNO_3, were inoculated with C. pyrenoidosa and cultured for ten days. At the end of the growth period, 300 ml of each culture were adjusted to pH 3.0 with 0.1 N HCl and harvested. The cells harvested from the urea culture were adjusted to pH 6.0 with 0.1 N NaOH, as was the liquor recovered from the harvester. The cells were resuspended in a medium composed of 250 ml of liquor, 100 ml of fresh urea medium, and, to maintain a constant 700 ml culture volume, 430 ml of deionized-distilled water. (The effect of this dilution of the medium will be discussed later). This procedure was repeated for nine harvest-growth periods totaling 51 days.

The culture grown in KNO_3 medium was treated in the same manner with the exception that 100 ml of fresh KNO_3 medium and the liquor from the harvest of the KNO_3 cells were used, in turn, to reconstitute the culture. Fresh urea and KNO_3 comparison cultures were inoculated with fresh cells at the start of each growth period. These cultures contained no liquor or deionized-distilled water, and were cultured under normal conditions in order to provide a culture against which the response from harvested cells could be compared. Packed cell volume (pcv) was recorded at the beginning and end of each growth period for the experimental and comparison cultures.

TABLE 1

VIABILITY OF CELLS HARVESTED FROM UREA OR KNO_3 MEDIUM WHEN RESUSPENDED IN LIQUOR MEDIUM SUPPLEMENTED WITH EIGHTEEN PERCENT FRESH MEDIUM

		Percent PCV Compared To	
Culture	PCV* (ml/ml)	Urea	KNO_3
Urea Comparison**	.0038	100.0	124.4
KNO_3 Comparison	.0031	80.3	100.0
Harvested Urea Cells	.0031	81.1	100.9
Harvested KNO_3 Cells	.0024	68.5	78.4

*Average net pcv of nine growth periods.

**Comparison cells were not harvested, and were grown in fresh, complete media.

Average harvest values for nine growth-harvest cycles (Table 1) show that the urea supported more growth than the KNO_3 in the complete medium as well as in the liquor medium. The growth in the liquor-urea medium was about the same percentage of the urea comparison as the growth in the liquor-KNO_3 medium in relation to its comparison culture. The general conclusions to be drawn are:

(1) The cells which were subjected to continuous culturing through nine harvests over 51 days remained viable;

(2) Overall, urea cultures supported better growth (as evidenced by harvested pcv) than the KNO_3; and

(3) Although the complete medium supported more growth than the liquor medium, the growth in recycled and reconstituted liquor medium was sufficient to warrant its consideration in continuous culturing procedures.

In order to determine the viability of cells which were continuously recycled, and resuspended in fresh medium, and to compare these cultures to cultures of fresh cells in fresh medium, the following determinations were made.

Seven hundred ml of urea medium were inoculated with C. pyrenoidosa and cultured for ten days; 300 ml of the culture were adjusted to pH 3.0 with 0.1 N HCl and harvested. The harvested cells were adjusted to pH 6.0 with 0.1 N NaOH, brought up to 100 ml with deionized-distilled water, and divided into two equal portions. One 50 ml portion was reconstituted to 700 ml with fresh urea medium, and the other 50 ml portion was used in the study on growth in harvest liquor. The portion reconstituted with fresh medium was grown for a period of time after which 300 ml were harvested. The harvested cells were adjusted in pH and resuspended to 700 ml in fresh urea medium.

The harvest-growth cycle was repeated five times during the experiment with the only variable being the length of the culturing period. Fresh comparison cultures were inoculated at the beginning of each growth period. Packed cell volumes were recorded at the start of each growth period and again when the culture was harvested.

The pcv and percentage relationship of the harvested culture and the comparison culture are presented in Table 2.

Table 2 shows that, on the average, harvested cells achieved essentially the same growth as the comparison cultures. Furthermore, it is obvious that the recycling through five harvest-growth periods did not result in the loss of viability of harvested cells resuspended in fresh medium.

The extent to which fresh algal cells are supported by liquor that has been used repeatedly for growth-harvest cycles was also determined. A 500 ml aliquot of urea medium was inoculated with C. pyrenoidosa, cultured for four days, and harvested. The liquor obtained from the harvest was adjusted from pH 3.0 to pH 6.8 by the addition of 0.1 N NaOH and inoculated with a known concentration of fresh algal cells. This cycle was repeated for six harvest-growth periods with the only variation being the length of the individual growth periods. No fresh nutrients, liquor, or deionized-distilled water was added to the original liquor. For comparison, at the beginning of each growth period, fresh urea medium was inoculated with fresh algal cells of the same concentration as introduced into the liquor culture. The comparison cultures were grown and harvested under normal conditions.

It was found that the liquor does support growth of the algae, but not so well as the fresh medium. Whether the smaller amount of growth in the liquor cultures results from a depletion of nutrients or a build-up of auto-inhibitory metabolic products was further investigated.

Ability of Harvest Liquor to Support Growth

Unless a thorough analysis is made to anticipate the exact amount of nutrients required, media compounded for algal culturing normally contain an excess of nutrients. When froth flotation is used to harvest the cells from such cultures, the majority of the excess nutrients remain in the harvest liquor, and, if this liquid is discarded, the nutrients are wasted.

The results presented in Table 1 indicate that harvest liquor can be recycled. However, the cultures in these studies were supplemented with fresh medium, and the extent to which the liquor alone will support growth cannot be determined. Therefore, to investigate the possibility of using the harvest liquor for the subsequent growth of cells; to determine the viability of harvested cells recycled in harvest liquor; and to determine the limit to which the harvest liquor, diluted by addition of deionized-distilled water with no supplemental medium added can support growth, the following experiments were conducted.

TABLE 2

VIABILITY OF CELLS HARVESTED FROM UREA MEDIUM AND RESUSPENDED IN FRESH UREA MEDIUM

	Harvested Culture			Comparison Culture*			
Growth Period Days	PCV (ml/ml) Inoculum	PCV (ml/ml) Harvest	Net PCV (ml/ml) Growth	PCV (ml/ml) Inoculum	PCV (ml/ml) Harvest	Net PCV (ml/ml) Growth	Percent Net PCV of Harvested Culture in Relation to Comparison Culture
4	.0005	.0030	.0025	.0010	.0040	.0030	83
5	.0005	.0050	.0045	.0005	.0050	.0045	100
4	.0013	.0045	.0030	.0015	.0035	.0020	150
5	.0007	.0050	.0043	.0007	.0045	.0038	112
6	.0005	.0020	.0015	.0005	.0025	.0020	75
Average	.0008	.0037	.0031	.0008	.0037	.0030	103

* Fresh, unharvested cells.

The 50 ml of cells set aside during the experiments on growth of recycled cells in fresh medium were introduced into a medium composed of 250 ml of clarified liquor and brought up to 700 ml volume with 400 ml of deionized-distilled water. The cultures were grown for various periods, and 300 ml were harvested each time. The harvested cells were adjusted in pH, and resuspended in a liquor-deionized-distilled water medium. The harvest-growth cycle was repeated five times throughout the experiment in progressively diluted liquor media with the length of the culturing period and level of dilution as variables.

Packed cell volume relationship, the percent relationship of liquor to deionized-distilled water, and the effects of cumulative dilution of the original liquor on subsequent growth are presented in Table 3.

TABLE 3

VIABILITY OF CELLS HARVESTED FROM UREA MEDIUM AND RESUSPENDED IN PROGRESSIVELY DILUTED LIQUOR MEDIA

	Sequential Growth Periods (days)				
Culture	4	5	4	5	6
PCV at Harvest	.0035	.0035	.0025	.0020	.0015
PCV Inoculum	.0003	.0008	.0010	.0004	.0005
Net PCV	.0032	.0027	.0015	.0016	.0010
% Original Liquor	35.70	12.75	4.56	1.62	0.58
Dilution Factor Original Liquor	2.8	7.8	21.9	61.8	172.3

The growth response of the culture shows decreased growth with increased dilution of liquor (Table 3 and Fig. 1). However, growth decreased less than might be expected from the dilution factors, since a 61.5-fold dilution (172.3/2.8) resulted in only a three-fold diminution of growth. This dilution does not take into consideration the nutrients removed from the liquor by previous growth. The response indicates that the nutrients were not completely depleted from the liquor during the 24 day period even though those nutrients were already considerably diluted at the beginning of each growth period.

By comparing the growth of the harvested culture grown on fresh urea medium (Table 2), with that of the harvested culture grown on diluted liquor

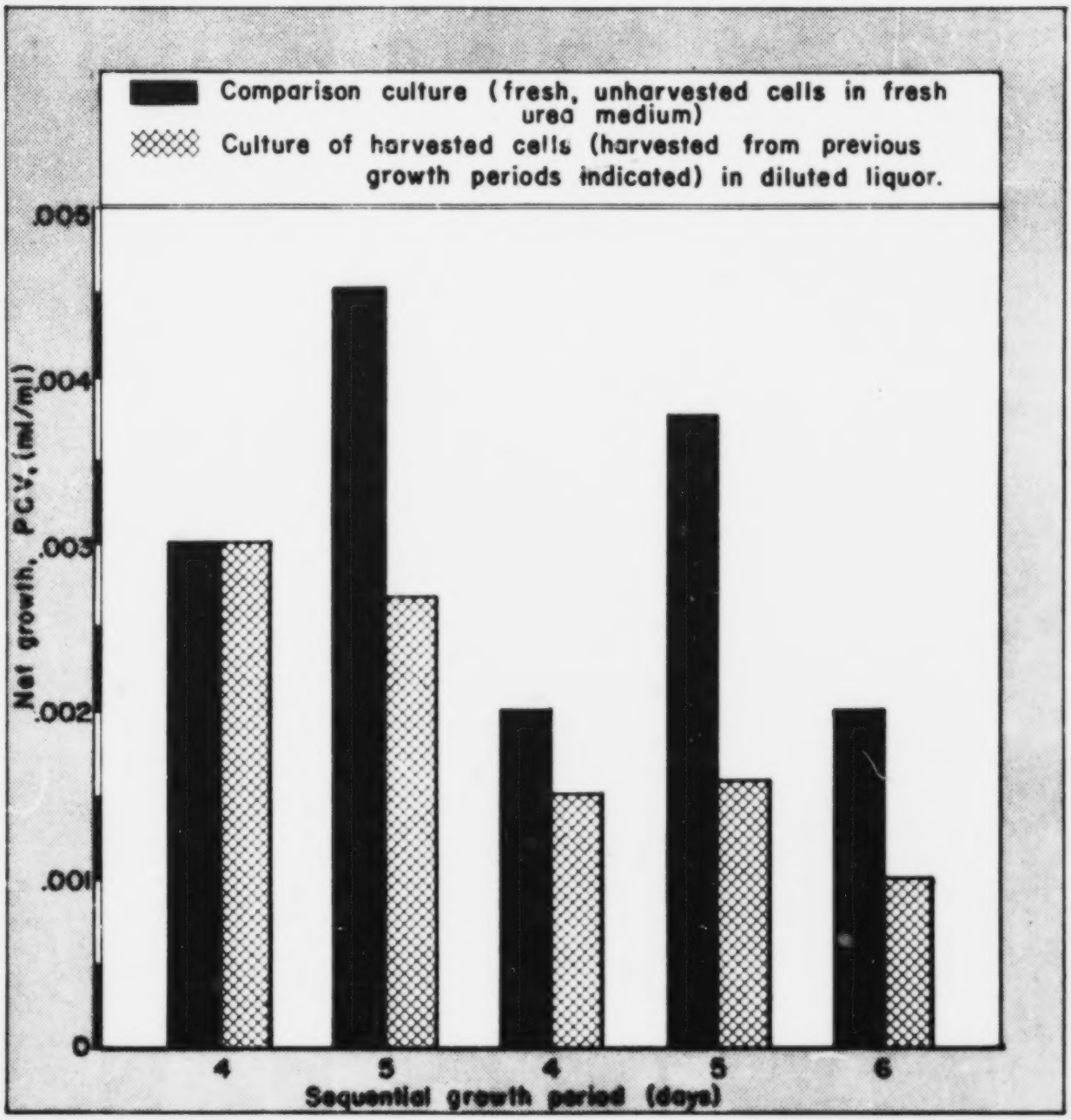

Figure No. I — Viability of cells harvested from fresh urea medium and resuspended in diluted liquor medium.

(Table 3), it is obvious that the liquor can be recycled, but it is equally obvious that the harvest liquor should be supplemented to support optimum growth.

Effect of Low pH on Viability

The following experiment was conducted to determine the viability and general condition of C. pyrenoidosa cultures held for a period of 24 hours at pH 3.0.

Two 400 ml Chlorella cultures, one supplemented with urea and the other with KNO_3, were cultured for five days. At the end of the five-day growth period, each culture was split into 200 ml portions. One portion of the urea culture was adjusted to pH 3.0 with 0.6 ml of 1N HCl, and one portion of the KNO_3 culture was adjusted to pH 3.0 with 4.0 ml of 1 N HCl. The other portion of each culture was retained at its normal pH, 6.4 for the urea and 9.5 for the KNO_3, and used as a control culture. The experiment was conducted at room temperature (22° C). Visual observations and photomicrographs of all cultures were made every two hours for the 24-hour period. At the end of the study, the experimental cultures were adjusted from pH 3.0 to pH 7.0. Each culture required 2.1 ml of 0.1 N NaOH, was brought up to 700 ml volume with the appropriate fresh medium, and was cultured for five days. The urea control culture was adjusted to pH 7.0 with 0.2 ml of 0.1 N NaOH, and the KNO_3 control culture was adjusted to pH 7.0 with 1.5 ml of 1 N HCl. The controls were brought up to 700 ml with the appropriate fresh medium and cultured for five days. Packed cell volumes and percentage relationship are recorded in Table 4.

TABLE 4

VIABILITY OF CELLS CULTURED IN UREA AND KNO_3 MEDIUM SUBSEQUENT TO MAINTENANCE AT pH 3.0 FOR 24 HOURS AS COMPARED TO NORMALLY MAINTAINED CONTROLS

Culture	PCV Inoculum	PCV 5 Days	Net PCV Growth	Percent PCV Compared to: Urea	KNO_3
Urea Control pH 6.4	.0010	.0060	.0050	100.0	76.9
KNO_3 Control pH 9.5	.0025	..0080	.0065	130.0	100.0
Urea pH 3.0	.0010	.0050	.0040	80.0	61.5
KNO_3 pH 3.0	.0015	.0060	.0045	90.0	69.2

The data in Table 4 suggest that some impairment of the viability of a Chlorella culture occurs when it is maintained at pH 3.0 over a 24-hour period. This was further confirmed by the visual observations in which some cell lysis and cellular debris were noted. However, the data also demonstrate that catastrophic losses did not occur in the experimental cultures. The urea experimental culture achieved 80.0%, and the KNO_3 experimental culture achieved 69.2% net growth in respect to their controls.

Clumping of the cells in the KNO_3 experimental culture was first observed during the second hour of the study and reached its maximum during the sixteenth hour after which the clumping appeared to diminish slightly. Minimal clumping occurred in the KNO_3 control culture after the twenty-first hour and minimal cell lysis and cellular debris were noted in the KNO_3 experimental culture after 12 hours, but they were not evident after the fourteenth hour.

Slight clumping occurred in the urea experimental culture during the second hour, and this clumping, as did the clumping in the KNO_3 experimental, reached its maximum during the sixteenth hour. Cellular debris appeared in the culture during the sixth hour and lysed cells were evident in the twelfth, eighteenth, and twentieth hours, but were few in number and were not observed after the twentieth hour. Minimal clumping occurred in the urea control culture.

Although cells of C. pyrenoidosa clump when they are subjected to pH 3.0, the data in Table 4 indicate that culture viability may be impaired but is not lost after cells have been subjected to pH 3.0 for 24 hours. This untoward effect would very likely be greatly attenuated in pH 3.0 exposure periods approximating those required for harvesting operations.

Determination of Harvest Efficiency

It was previously reported (1) that maximum froth production occurs when the pH of the harvest feed is adjusted to 3.0; therefore, no further analysis of effects of pH on harvest efficiency were conducted.

In previous studies made at Resources Research, Inc., harvest phenomena have been observed by measuring concentration of the harvest directly. There are some imposing weaknesses associated with this approach. First, it is difficult to collect all of the harvest foam; some of it adheres to the walls of the harvest cylinder and the discharge tube. Second, there is generally a "first-burst" of foam, denser in algal cells than the foam subsequently

driven from the harvest feed suspension. Third, foam near the foam-feed liquid interface is rather unstable and readily bursts during collection attempts, causing cells previously foamed free to return to the harvest feed suspension.

Manipulation of the collected harvest and the difficulties associated therewith could be avoided if reliable indirect determinations could be made, based on measurements of the feed suspension cell content before and after a harvest operation. By calculating the difference between these values, the amount of cells removed from the feed culture could be determined.

The Standard Methods (4) Jackson candle determination of turbidity was explored as a means of determining cell concentrations in the harvest feed suspension.

It was necessary to determine if a direct relationship between cell concentration of sample and turbidity value existed. A series of cell dilutions was prepared. Comparisons between turbidity readings and pcv's were made, and a direct relationship was found. The standard curve for these values appears in Fig. 2. The Jackson candle method was found to be a reliable, rapid method of determining the density of algae in harvest feed suspensions.

Determination was made of the aeration rate and aeration exposure time necessary for the most economical use of air during the flotation harvesting process. Three acrylic harvest columns were prepared with sintered glass sparges attached to the bottom. Air lines were connected to each sparger-column assembly and in-line air-flow meters were calibrated to deliver air at rates of 40, 90, and 155 cc/min at 15 psi.

Cultures were grown in one-gallon jugs for five to seven days in the medium previously described.

The pcv's of the feed cultures used ranged from 0.0026 to 0.0034 ml/ml. Jackson candle determinations were made on the harvest feed cultures prior to acidification. After pH adjustment to 3.0, the feed suspensions were subjected to aeration periods of 1, 3, and 15 min at each of the three aeration rates. After each independent harvest trial, the turbidity of the harvest feed liquor was determined, and then the amount of harvest was calculated.

There was some concern that carry-over of water with the harvest froth might alter both the concentration of harvest and volume of the feed, especially for samples exposed to the highest aeration rate for 15 min. Therefore, periodic indirect measurements of the volume of harvest concentrate were made. This was done by measuring the volume of feed suspension remaining

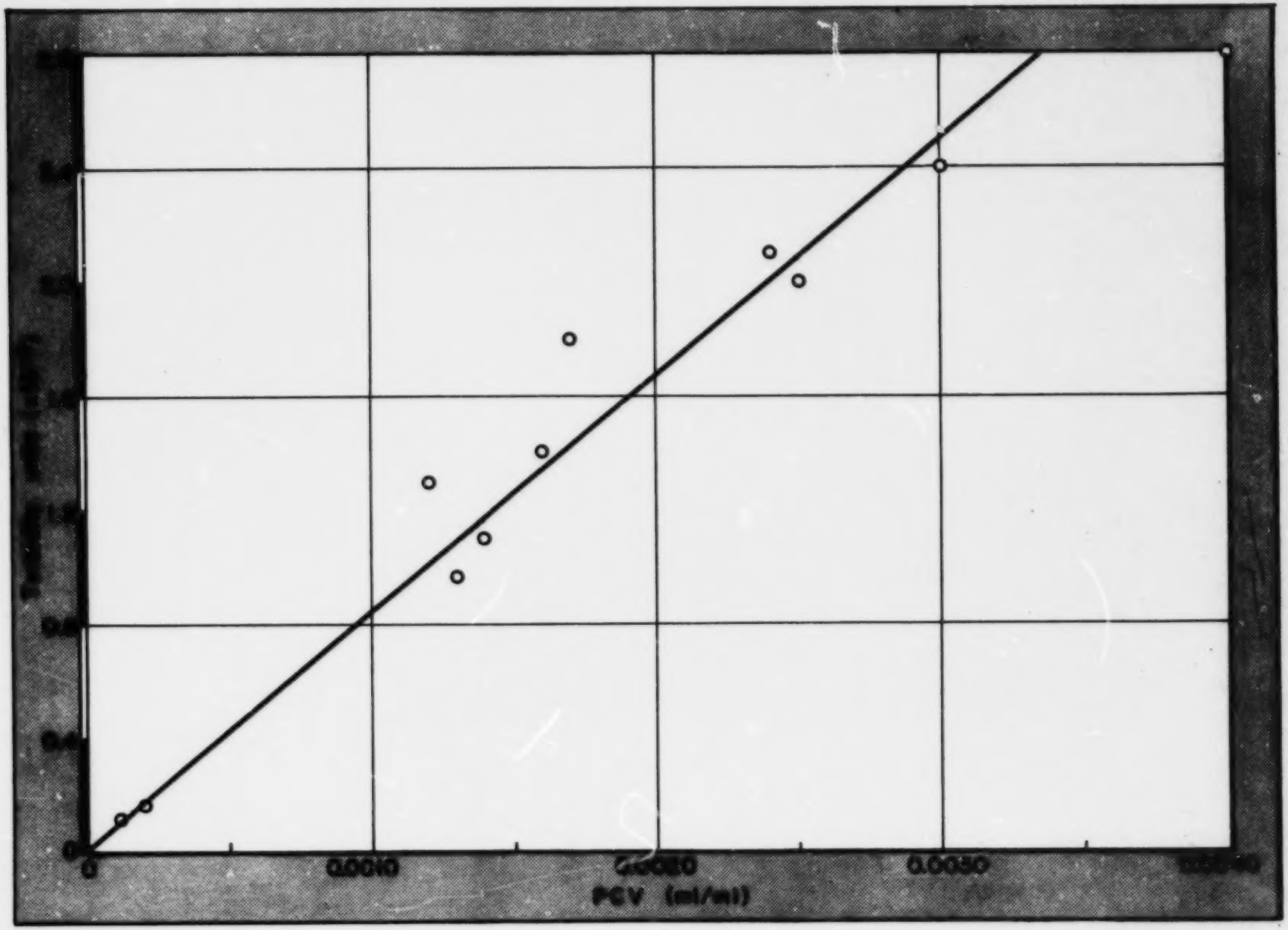

Figure No. 2 — Standard curve: correlation between turbidity and PCV of algal suspensions.

in the harvest column after harvesting, and the decrease due to carry-over with the harvest was calculated. Post-harvest volume of the feed was found to have decreased by only 1-2 ml. Water carry-over therefore had negligible effect on harvest concentration and cell concentration in the feed. Harvest concentration factors ranged from 162 to 485, with the mean being 346. Therefore, harvest concentration factors reported here are considerably higher than concentrations reported previously (1) which only occasionally reached 200-fold.

The percent harvest at each aeration rate in relation to length of the aeration period is shown in Table 5. At all aeration rates, the cumulative percentage of each culture harvested increased with time. Little additional

TABLE 5

PERCENT HARVEST AS A FUNCTION OF DURATION OF AERATION AND AERATION RATE

Duration of Aeration, (min)	Percent Harvest at Indicated Aeration Rate*		
	40 cc/min	90 cc/min	155 cc/min
1	56.7	68.7	88.8
3	68.4	87.9	97.2
15	92.4	88.6	98.5

*Average of 3 replications

harvest occurred after three min of aeration at 90 and 155 cc/min, but an appreciable harvest was obtained by continuing harvesting beyond three min at 40 cc/min. The data, as presented here, indicate that the aeration rate of 40 cc/min is the most inefficient in terms of exposure time. It is also evident from Table 5 that the most efficient harvesting occurs at the highest aeration rate, 155 cc/min.

The amount of harvest relative to unit of air expended during the three periods of aeration is important to the overall economy of the system. This relationship is shown in Table 6. Percent harvest per unit of air expended was greatest when the harvest feed was aerated at 40 cc/min, for all equal aeration periods compared. In fact, the amount of harvest per unit of air expended at 40 cc/min was at least twice that for each of the two higher aeration rates when equal aeration periods are compared. Percentage harvest per unit of air decreased as the aeration rate was increased. This is in agreement with a

TABLE 6

PERCENT HARVEST PER UNIT OF AIR AS A FUNCTION OF AERATION RATE AND LENGTH OF AERATION TIME

Duration of Aeration (min)	Percent Harvest per cc of Air at Indicated Aeration Rate		
	40 cc/min	90 cc/min	155 cc/min
1	1.42	0.76	0.57
3	0.57	0.33	0.21
15	0.15	0.07	0.04

previous report (1) which stated that harvest concentration increased sharply as the aeration rate was decreased. These data also show that for all aeration rates, the rate of harvest is drastically reduced when feed suspensions are aerated for more than 3 min. Therefore, on the basis of air expenditure for desired percent clarification, the most economical harvesting of algae occurred when cultures of densities considered here were aerated at 40 cc/min for three min. Additional work should explore the 15 min harvesting interval by finder increments to determine the optimum period of aeration more closely. Furthermore, the evaluation of the optimum aeration rate and aeration period for harvesting on the basis of percentage harvest (Tables 5 and 6) has value only where comparable feed densities are considered.

Culture Density and Harvesting Economy

The minimum and maximum culture densities that could be economically harvested were investigated. An experiment was designed such that daily harvest determinations could be made on cultures grown in one-gallon jugs for one to five days, and initiated at pcv's of 0.0005, 0.0010, 0.0020, and 0.0040 ml/ml. Initial culture density and culture age at time of harvest could be evaluated from the standpoint of harvest efficiency and economy. The cultures were incubated at 37$\pm$ 2^{o}C under approximately 2000 ft-c illumination, and aerated with a mixture of approximately three percent CO_2-in-air. On the basis of results described above, all feed cultures were flotation harvested for three min at an aeration rate of 40 cc/min. Harvests were determined indirectly by the Jackson candle method.

Initial culture densities for all except pcv 0.0040 were achieved by diluting three to five day old stock cultures with approximately equal volumes

of the previously described fresh urea medium. The pcv of the stock cultures was too low to obtain a pcv of 0.0040 by simple dilution. To achieve this initial pcv, 20.09 g of algal cells were centrifuged from about seven l of three to five day old stock cultures and diluted with a medium composed of equal volumes of filtered stock culture harvest liquor and fresh urea medium. The actual pcv of the resultant culture was 0.0041 ml/ml. Froth flotation harvesting was advantageously used to reduce centrifuging effort. Although exposure of cells to pH 3.0 has little adverse effect on viability, the harvest concentrate was periodically neutralized with 0.1 N NaOH. The preparation of this culture was inconsistent with the dilution method used for the lower culture densities, but otherwise this concentration could not be obtained. Growth rates of cultures prepared by the two methods were compared in a preliminary experiment. Stock culture and centrifuged cell dilutions were prepared to give a calculated initial pcv of 0.0010 ml/ml. Stock cultures were diluted to the pcv with an approximately equal volume of fresh urea medium. Centrifuged cells were added to a medium composed of equal volumes of fresh urea medium and neutralized harvest liquor so that cells prepared by the two methods would be grown in comparable media. Growth curves based on pcv are compared in Fig. 3. It therefore appeared valid to compare harvest data for cultures grown from centrifuged cells with harvest data from the other three culture densities.

The amount of cells obtained for preparation of the cultures of a pcv of 0.0040 ml/ml was insufficient to provide culture volumes that could be sampled daily. Therefore, harvest determinations were made after one, two, and four days of growth.

The influence of age of culture and culture density at the time of harvest on harvest efficiency is shown in Table 7. Regardless of initial culture density, the efficiency of cell removal decreased with culture age. Harvest efficiency for the most dilute culture (initial pcv, 0.0005 ml/ml) was greater than that of the higher culture densities, regardless of culture age at the time of harvesting.

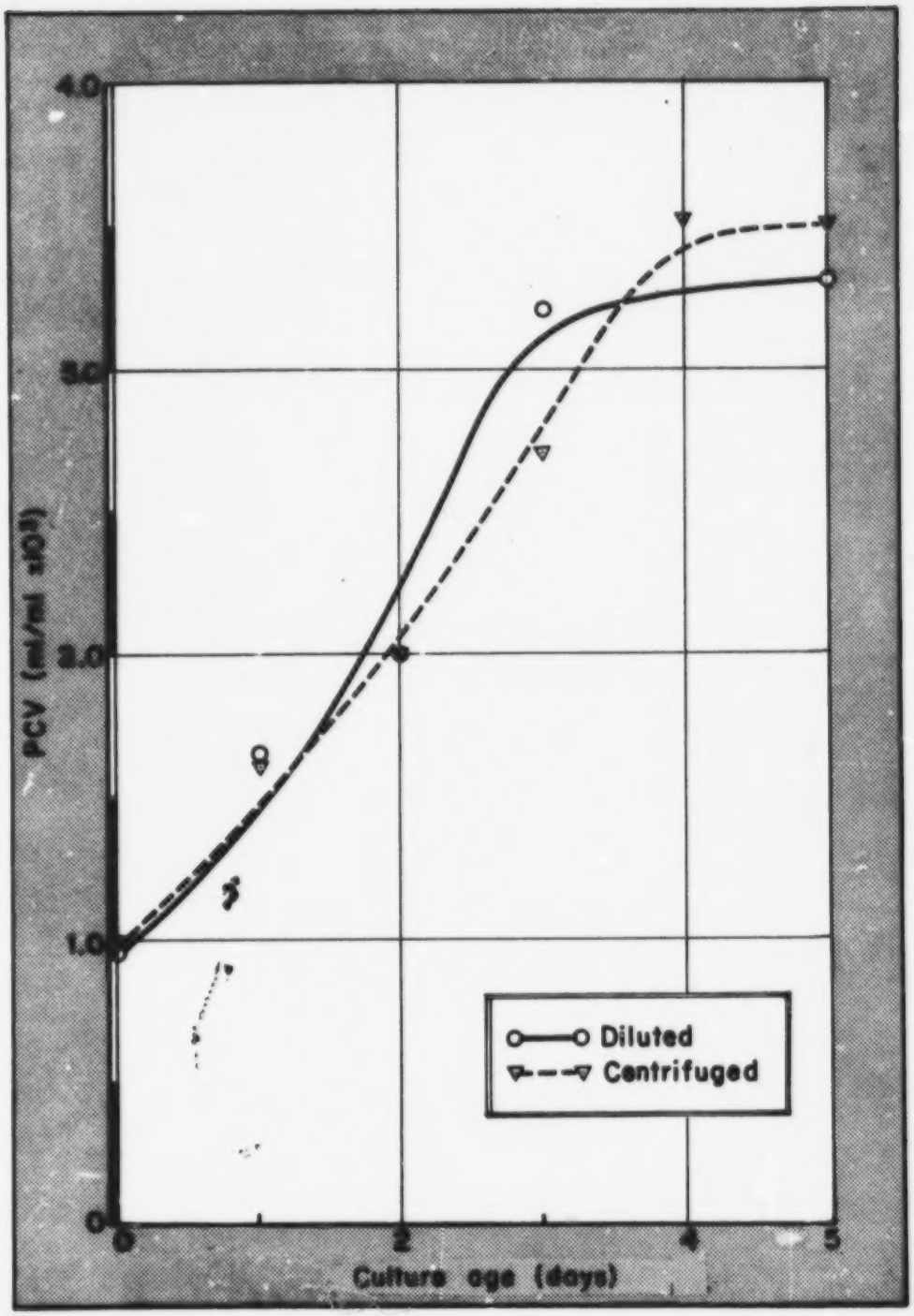

Figure No. 3 – Growth comparison: centrifuged cells vs. diluted stock culture; both diluted to equal starting densities.

TABLE 7

RELATIONSHIP OF INITIAL CULTURE DENSITY AND CULTURE DENSITY AT HARVEST TO HARVEST EFFICIENCY

Initial Culture Density, pcv	Percent Harvest at Indicated Culture Age, days *				
	1	2	3	4	5
0.0005	97.4	89.7	81.3	78.4	62.5
0.0010	67.1	69.2	65.1	60.0	42.5
0.0020	81.5	82.6	71.5	54.7	54.0
0.0041	62.9	85.6	-	30.9	-

* Average of 3 harvest trials.

The product of harvest efficiency (Table 7) and the pcv of the non-acidified culture at the time of harvest is the amount of algae removed from the feed. In Fig. 4, the amount of harvest is plotted as a function of the age of culture at the time of harvest for each initial culture density. Cell removal was greatest between about 3.5 and 4.5 days of growth for the cultures initiated at 0.0005 and 0.0010 ml/ml. For cultures initiated at a pcv of 0.0020 ml/ml, maximum harvest was obtained between two to three days of growth. A very high cell harvest was obtained for cultures initiated at a pcv of 0.0041 ml/ml after two days of growth. As initial pcv was increased from 0.0010 to 0.0041 ml/ml, the growth interval for maximum harvest decreased. These results indicate interactions among culture density, absolute amount of harvest, and harvesting efficiency. Harvesting efficiency data alone would provide no information on the actual amounts of cells removed from these suspensions.

Conceivably, an inoculum level higher than 0.0041 ml/ml might be reached whereupon froth flotation efficiency would break down and harvest levels would fall to those of more dilute cultures. From Table 7 and Fig. 4, harvest efficiency as well as cell removal did fall dramatically with time for pcv 0.0041 cultures after four days of growth, indicating that, after this age, the ability of the frothing agent to foam free cells did not increase in proportion to growth of the cultures. However, experimental limitations described above did not permit observation of a harvest crossover point. Extrapolation of the curve for the highest culture density indicates that a harvest crossover would have occurred at about five days of growth.

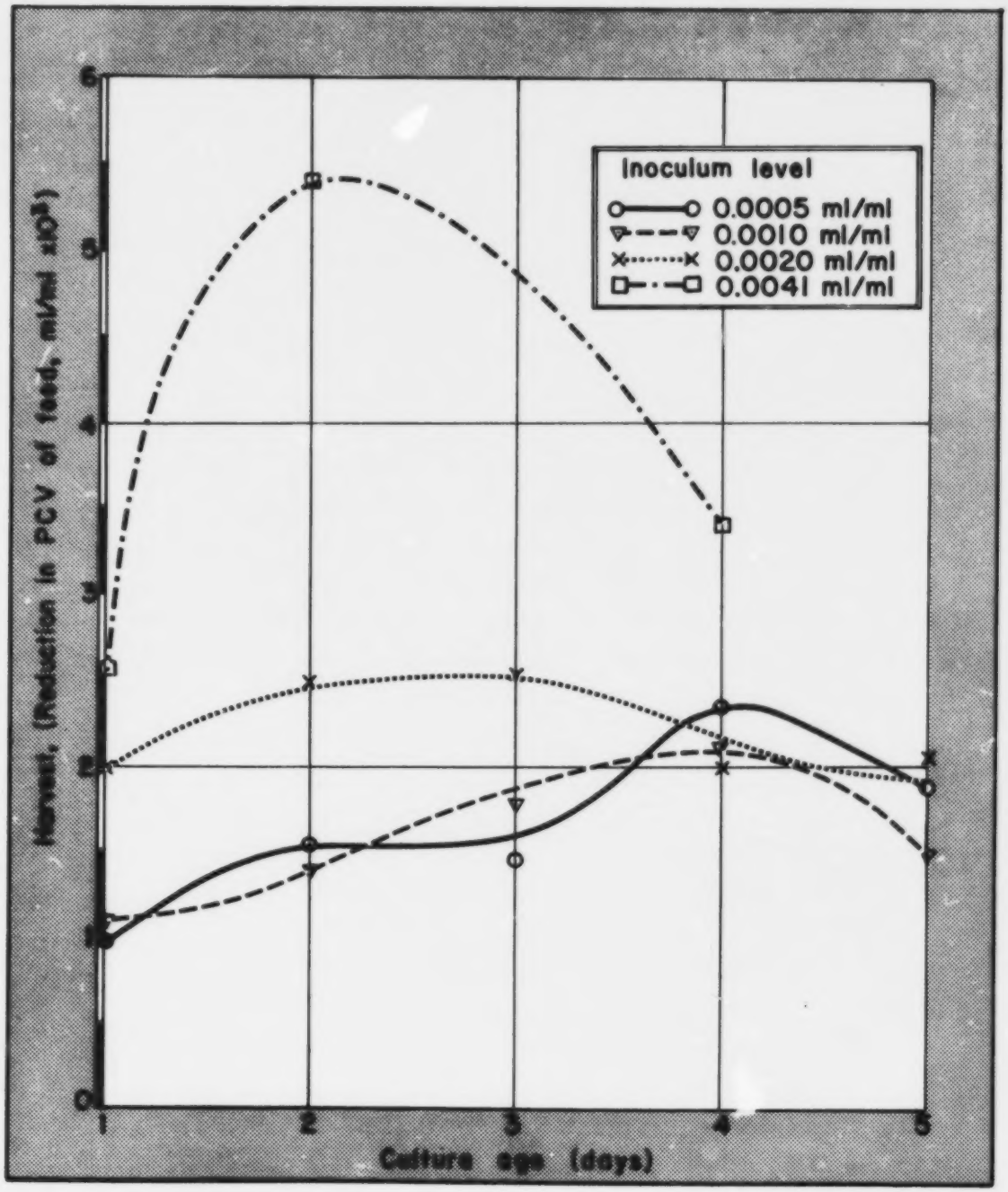

Figure No. 4 — Harvest from cultures of various initial densities as a function of age at time of harvest.

If culture age is ignored, and the amount of algae in feed suspensions is plotted against amount of algae removed, a region of diminishing harvest relative to increasing feed culture density becomes apparent (Fig. 5). For the range of culture densities used in these studies, it appears that a maximum culture density for proportionate return in harvest lies at a value somewhere between 0.0063 and 0.0105 ml/ml. In other words, a maximum harvest accrued from cultures initiated at a pcv of 0.0041 ml/ml and grown for a period of about two days under conditions described above.

The data of Figs. 4 and 5 indicate that a minimum culture density for efficient harvesting and for total harvest was not achieved, although it seems certain to exist. The amount of harvest observed for cultures initiated at a pcv of 0.0005 ml/ml was not appreciably different from that of cultures initiated at a pcv of 0.0010 ml/ml, regardless of age of culture at harvesting.

In the above investigation, samples of each culture were acidified to a pH of 3.0 immediately preceding froth-harvesting operations. The culture pH and amount of 1N HCl required to adjust the 450 ml samples to pH 3.0 were recorded. In Table 8, acid requirements are expressed as ml of acid per liter of culture.

TABLE 8

Ml 1N HCl REQUIRED TO ADJUST 1 LITER CULTURES TO pH 3.0 AS FUNCTION OF CULTURE AGE AND CULTURE DENSITY

Initial Culture Concentration (ml/ml)	Acid Required* at Indicated Culture Age (days)				
	1	2	3	4	5
0.0005	2.70	4.66	5.17	8.15	11.41
0.0010	10.97	14.60	16.60	18.30	20.00
0.0020	12.40	14.07	16.17	18.00	21.98
0.0041	4.46	8.14	-	15.70	-

*Average of triplicates

Data presented in the above table show that, regardless of culture inoculum level, acid requirements increased as the age of culture at the time of harvest increased. This phenomenon might be due to the build-up of culture metabolites having high acid-buffering capacity. These data also show that, regardless of age of culture at time of harvest, cultures initiated at pcv's of 0.001 and 0.002 ml/ml required more acid than those initiated at the higher and lower

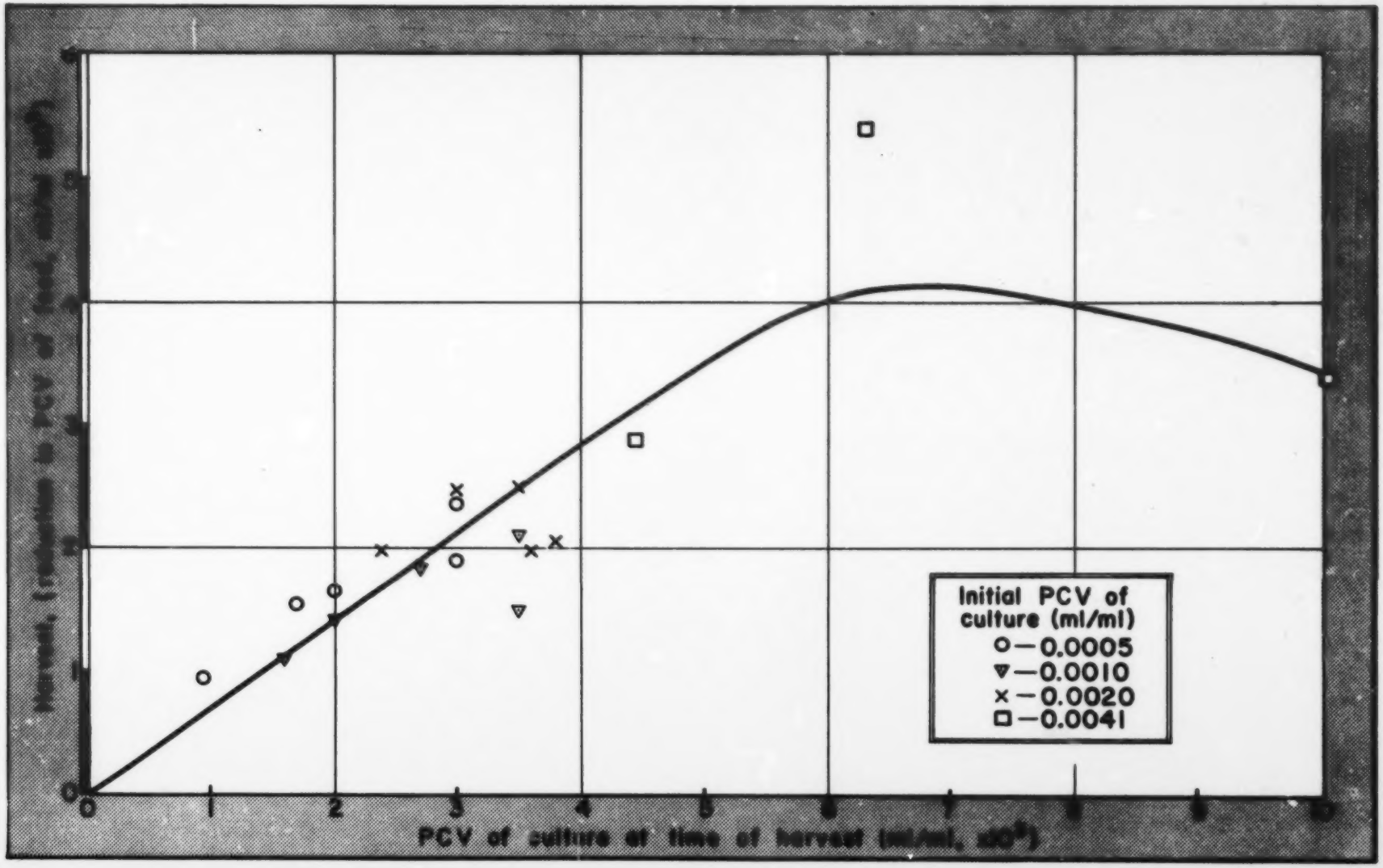

Figure No. 5 — Relation of harvest to culture density.

culture densities. By comparing the acid requirements for cultures initiated at a pcv 0.0005 ml/ml with those for cultures initiated at 0.0041 ml/ml, it can be seen that about two times more acid was required for the latter than for the former. However, when acid requirements on the basis of fresh weight of algae harvested were computed (Table 9), acid economy was greatest at the highest culture density. Here, as in Table 8 above, acid requirements for the two intermediate inoculum levels were greater, regardless of age of culture, than for the higher and the lower inoculum levels.

TABLE 9

Ml 1N HCl REQUIRED TO ADJUST CULTURE TO pH 3.0
PER GRAM OF FRESH CELLS HARVESTED

Initial Culture PCV (ml/ml)	Acid Required at Indicated Culture Age, (days)				
	1	2	3	4	5
0.0005	2.84	2.74	2.59	2.72	3.82
0.0010	6.68	7.30	6.15	5.24	5.72
0.0020	5.17	4.69	4.62	5.00	5.78
0.0041	0.97	1.29	-	1.50	-

Mass Culturing of Algae

Before unicellular algae can be efficiently grown and harvested over prolonged periods in continuous mass culturing and harvesting systems, factors conducive to maximal growth and harvest must be defined and quantified. Froth-flotation harvesting, integrated with a continuous mass culturing system, appears to be a promising means of reducing the cost and effort of mass culturing and mass harvesting operations.

Growth of algae in a mass culture system designed by Resources Research, Inc., has been studied during the current year. Preliminary studies on growth of mass cultures in defined media (p. 8) showed that some algae settle from suspension and form a dense mass on the rounded sides and bottom of the tank. Some improved means of agitating the culture medium was indicated and the gas sparger was redesigned from a single stainless steel sparger to a perforated tygon tube arranged in four spirals around the inner surface of the rounded bottom.

The sparger was tested in a growth study in which a dilute algal suspension of C. pyrenoidosa (711-05) was grown in urea medium at 39° C ± 1° C., illuminated by white light at about 2000 ft - c., and aerated with 5 percent CO_2 -in-air. The pcv of the culture increased five-fold by the middle of the second day, but remained at that value for the remainder of the 86-hour study. Periodic microscopic examinations of the culture showed that cell division was continuing. It was concluded that cells were settling from the medium as fast as new cells were being formed, and that the redesigned sparger could not prevent algal cells from settling. Visual observation revealed a thick mass of viable cells on the bottom of the tank.

A subsequent experiment on growth in mass culture, similar to the one above except that a higher initial culture pcv (0.0008) was used, showed a linear decrease in culture pcv between the first and fifth day of culturing. Again visual observation confirmed that cells were settling from suspension.

A low concentration (0.025% w/v) of glucose was added to the culture at this point in the study to examine the effects of supplemental carbon in organic form upon growth and settling. The pcv of the culture rose from 0.0005 to 0.0009 during the following 24 hours and remained at this level until the eighth day. By the ninth day the pcv had decreased to 0.0008 and remained so until the eleventh day. At this point, 0.025% glucose (w/v) was added and the pcv increased to 0.0020 within 24 hours. By the thirteenth day the pcv was 0.0029, and on the fourteenth day had decreased to 0.0028; at this time the experiment was terminated. The first glucose supplement resulted in a doubling of pcv in 24 hours and the second supplement resulted in an approximate four-fold increase in pcv within 48 hours.

Three other experiments were conducted on the effect of glucose supplementation on growth of mass cultures ranging in initial pcv from 0.0001 to 0.0006. Glucose was added on twelve occasions at intervals of from seven to 72 hours during these experiments and, on eleven occasions, growth response rates were proportional to the interval of time between supplements, regardless of the initial pcv of the cultures. On the one occasion where no response occurred, the culture pcv was 0.0016. After a period of 24 hours without response, glucose was added at double the above rates, and the pcv increased to 0.0024 within 16 hours. The culture pcv at the termination of this experiment (376 hours) was 0.0028. A thick

mat of algae was observed adhering to the bottom of the tank. To estimate the amount of algae that settled from the medium, the mat was scraped from the tank surface and mixed with an amount of water equivalent to the initial volume of the culture medium (114 l). The pcv of this suspension was 0.0075. If algal sedimentation had not occurred, the culture pcv would have been 0.0103 or 368% greater than that observed at the termination of culturing.

It was felt that, since algae adhered in dense mats to the flexiglass material comprising the culture tank, some material might be applied to the inner surface to reduce or eliminate adherence of the algae. Several materials of promise were obtained, and an experiment was designed in which duplicate samples of the test materials could be applied in various dimensions to the tank surfaces. The trade name, description, and method of application of the various materials tested appear below:

Trade Name	Description and Method of Application
1. Tygon Temp-R-Tape	1 x 6-in adhesive strips.
2. Tygon Film	1 x 6-in, 4 x 6-in, and 3 x 16-in adhesive strips.
3. Fluorocarbon Dispersion,* Experimental	Colloidal dispersion, painted on surface, covering 4 x 6- and 3 x 16-in areas.
4. Kel-F Fluorocarbon** Stopcock Grease	A grease spread on surface covering 4 x 6- and 3 x 16- in areas.

Culturing continued for seven days, at which time the pcv of the culture began to decrease, indicating sedimentation of cells. The culture medium was drained from the tank and the materials were examined for adherence of algae. A uniform mat was found adhering to all test materials, regardless of composition or surface area.

The materials as applied in these investigations, therefore, did not reduce the amount of sedimentation of algae in mass cultures. Some mechanical means of increasing the amount of agitation of the culture near the bottom of the tank might reduce or eliminate the amount of settling. This method remains to be investigated.

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*I. E. DuPont de Nemours and Company; Wilmington, Delaware.
**Minnesota Mining and Manufacturing; Minneapolis, Minnesota.

In all experiments, culture pH rose from initial values of 6.4-6.6 to terminal values of 7.3-8.1, depending on length of the culturing period. Mass culturing of algae as described here was conducted under non-sterile conditions. Since growth of bacterial contaminants is generally enhanced in alkaline conditions, and retarded in acid conditions, these organisms may have proliferated in late stages of the above unbuffered mass culturing experiments due to the onset of alkaline conditions. It is possible that growth of bacteria might have affected algal sedimentation. Growth of algae in partially open mass culture might require the incorporation of buffer systems to maintain pH conditions that minimize bacterial growth and do not affect agal growth.

Harvesting Costs

The present state of the art does not permit accurate determination of the cost of harvesting algae by froth flotation, but estimates are possible on the basis of costs of certain important steps in the operation. Capital cost estimates were not made; engineering design effort and pilot plant studies are necessary before such costs can be obtained.

The cost of adjusting a dilute culture and a relatively dense culture was calculated on the basis of certain acid requirements presented in Table 9. The amount of HCl required to lower the pH to 3.0 of the one-day old culture initiated at pcv 0.0005 and the two-day old culture initiated at pcv 0.0041 was the basis for this determination. Requirements of base for neutralization were not determined, so estimates of the cost of NaOH were based upon a ratio calculated from acid and base costs estimated by Levin et al (1). They estimated the acid and base costs at approximately 40 and 11 dollars, respectively. Based upon current prices of 20°Be' HCl and solid NaOH (76%) the cost of acid and base per ton of dry algae harvested from the dilute culture would be about 42 and 12 dollars, respectively. The cost of acid and base per ton of dry algae from the dense culture would be about 21 and six dollars, respectively.

Air costs were estimated, based on a cost of 0.005 dollars per 1,000 ft^3 at 7.25 psi for aeration of municipal sewage (5). The air cost for harvesting the dilute culture would be $.19 and that for the dense culture would be $.03 per ton of dry algae. This is a reduction of approximately four dollars per ton over air costs previously reported (1). This is because of the shorter aeration period and lower aeration rate.

Gotaas and Golueke (6) estimated the cost of commercial drying of algae at 20 dollars per dry ton. Total costs for producing one ton of dry algae would, therefore, be approximately 75 dollars for dilute cultures and approximately 47 dollars for relatively dense cultures. The value of dried algae was estimated to be 80 to 100 dollars per ton, and on this basis, a figure of merit of 35 to 40 dollars for economically harvesting a ton of dry algae was presented in 1957 (2). If these estimates are updated to compensate for increase in the Consumer Price Index since 1957, the current figure of merit would be 38.2 to 43.5 dollars. On the basis of the cost analysis made here, the figure of merit for producing a ton of dry algae has been virtually attained.

REMOVAL OF ALGAE AND SUSPENDED SOLIDS FROM SEWAGE LAGOONS

Preliminary Experiments

Two sewage lagoons, both at Hazleton Laboratories, Inc., Falls Church, Virginia, were investigated. Approximately 300 workers and 2,000 dogs and primates are served by the ponds which also receive chemical laboratory wastes. Initially a small, older pond was studied. In sampling the smaller pond, it was observed that the unadjusted sample foamed readily with some clarification of the sample. The foam at unadjusted pH (9.3) levels was thought to be due to detergent present. When the sample was adjusted to pH 3.0 with 1 N hydrochloric acid, both the algae and the detritus were removed from the feed suspension, although the harvested material clung to the walls of the harvest column. The harvested material was a sticky gelatinous mass. When the "detergent" was removed by pre-aeration at unadjusted pH, the pretreated sample, acidified to pH 3.0, could be harvested clear of the feed. Thus, pH control made it possible to "foam fractionate" the sewage.

The larger lagoon (273 ft x 273 ft x 28 in) was two months old at the initiation of the investigation. A design loading of 100 pounds BOD per day had not yet been achieved. The pH of the large sewage lagoon ranged between 7.5 and 9.5 with a mean average value of pH 8.5. Sampling was achieved by dipping a 5-gallon carboy into the lagoon; the carboy was transported to the laboratory where the harvesting experiments were performed.

Unadjusted samples from the large lagoon exhibited little, if any foaming tendency. This was attributed to the low "detergent" concentration. The parameters of aeration rate (40, 90, 155, and 220 cc/min) and pH levels (3.0, 3.5, and 12.0) were explored. It appeared that the higher aeration rates yielded

greater absolute concentrations of harvest, regardless of pH; however, as the aeration rate increased, more feed suspension was mechanically transferred to the receiving container and the harvest was diluted. Although it appeared that all of the detritus and algae was removed in most cases, the feed suspension adjusted to pH 12.0 and aerated at 155 cc/min produced the greatest concentration of harvest. The variability of observed results and measured values was thought to be due to variation in the amount of harvest adhering to the walls of the harvest column between the feed level and the discharge orifice. The higher values at pH 12.0 were thought to be due to the great stability and amount of foam produced at this pH. A more thorough washing of the harvested material from the column was achieved than at the lower pH values.

During attempts to measure the amount of solids in sewage samples by determination of the packed cell volume, it was observed that solids clung to the shoulders of the centrifuge tubes to varying degrees, thereby making the packed cell volume method unreliable. Thus, it was difficult to get a representative sample of harvest, and the pcv was not easily determined. This lack of precision in measuring the concentration of the algae and detritus in sewage samples indicated a need for a more precise method of measurement. Therefore, two methods, the Gooch method of non-filterable solids (7) and the Jackson candle turbidimetric method (4) were investigated. Although the method of non-filterable solids was quite sensitive to small changes in the suspended solids concentration in the sewage sample, the method was discarded in favor of the more rapid turbidimetric procedure. The turbidity values determined by the Jackson candle method were standardized against the non-filterable solids determinations (Fig 6). For the samples tested, a linear relationship existed between weight of suspended non-filterable solids and turbidity. The Jackson candle method, therefore, was found to be sufficiently reliable for determining the amount of solids suspended in sewage lagoon samples. Therefore, all subsequent harvesting procedures on sewage lagoon samples were evaluated in terms of turbidimetric units.

Clarification Variables and Indices

Although the percentage clarification index is the generally accepted one used to describe sewage treatment efficiency, it is not very meaningful here. Moreover, these studies have caused an examination of the index which makes it

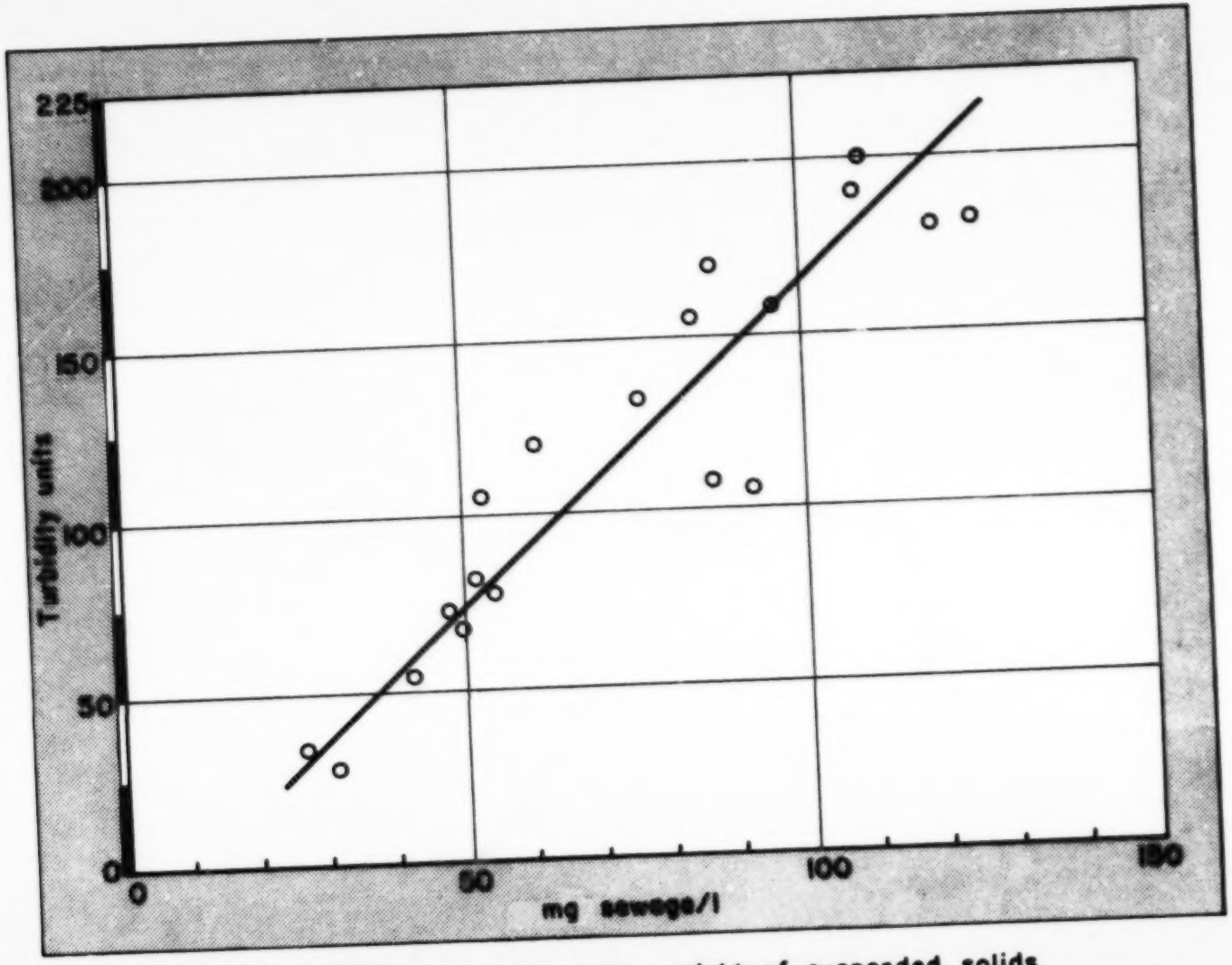

Figure No. 6 — Standard curve, dry weight of suspended solids vs. turbidity of sewage.

appear to be of questionable value in sewage treatment also. A process which removes a high percentage of suspended algae or sewage particulates from a dilute suspension may not be very efficient in terms of harvesting or sewage treatment objectives. On the other hand, a process removing only a small percentage of a dense suspension may be far more relevant to the process objectives and, hence, more efficient from the standpoint of the objectives. The best way to characterize clarification processes would seem to be in terms of absolute accomplishment, i. e., amount of material removed per unit of effort, materials, or time. The selection of the most suitable process or range of operation would then be made on the basis of the specific need.

Effect of pH on Clarification of Lagooned Sewage

Acrylic harvest columns measuring 55.9 cm high with an inside diameter of 3.8 cm were used to harvest samples taken from the sewage lagoon. The discharge orifice of the column was situated 5.5 cm below the upper rim. A feed suspension of 450 ml filled the column to a point 7.6 cm below the discharge orifice. A single sintered glass sparger 3.1 cm in diameter with a porosity of five microns was used to bubble air through the feed suspension in each column.

Four hundred and fifty ml portions of sewage lagoon feed suspensions were adjusted to pH 3.0, 3.5, and 12.0. The feed suspensions were aerated at approximately 40, 90, and 155 cc of air/min, and tested at each pH value. Initially, the time at which a first burst of harvest occurred was recorded. Visually, it appeared that, after the first three min of aeration, little additional clarification was achieved. A time interval mid-way between the start of aeration and the three min clearing time was chosen to obtain data points for recording short term rates of clarification. Therefore, a "mid-point" value of 90 sec was chosen. The results are presented in Table 10.

From the data, it is apparent that amount of clarification at pH 3.0 and 12.0 is equal. The choice of pH, then, is a matter of economics. Since the results are equal, the lowest cost to reach the desired pH level would determine which pH level to utilize. On the basis of costs, pH 3.0 is the level of choice since, from experimental data, pH 3.0 is achieved at a cost of $28.40 per million gallons of lagooned sewage versus $278.00 per million gallons adjusted to pH 12.0. The acidification of the lagoon has an additional advantage over

TABLE 10

THE EFFECT OF pH ON THE FROTH FLOTATION CLARIFICATION OF LAGOONED SEWAGE

pH 3.0

Aeration Rate (cc/min)	40		90		155	
Time of Sampling Feed	Turbidity Units	Time (sec)	Turbidity Units	Time (sec)	Turbidity Units	Time (sec)
Initial Feed	170	0	170	0	170	0
First Burst	120	52	105	22	128	17
Mid-point	108	90	86	90	90	90
Clearing	76	180	73	180	67	180

pH 3.5

Aeration Rate (cc/min)	40		90		155	
Time of Sampling Feed	Turbidity Units	Time (sec)	Turbidity Units	Time (sec)	Turbidity Units	Time (sec)
Initial Feed	190	0	190	0	190	0
First Burst	188	47	182	32	188	44
Mid-point	155	90	180	90	150	90
Clearing	101	180	93	180	100	180

pH 12.0

Aeration Rate (cc/min)	40		90		155	
Time of Sampling Feed	Turbidity Units	Time (sec)	Turbidity Units	Time (sec)	Turbidity Units	Time (sec)
Initial Feed	170	0	170	0	170	0
First Burst	95	68	105	80	115	24
Mid-point	100	90	90	90	90	90
Clearing	76	180	84	180	66	180

the addition of alkali in that the acid is commercially available in a liquid form more readily measured and mixed with the feed suspension than the commercially available sodium hydroxide solid flakes. For these reasons, pH 3.0 was selected for further evaluation.

Over a period of several weeks, many experiments were performed to recheck the previously reported data. From these experiments, all of which were independent trials due to the destructive nature of the procedure, a compendium of all data at pH 3.0 has been prepared (Table 11). Turbidity values were converted to weight of suspended solids by use of the standard curve for sewage (Fig 6). Several striking phenomena were observed. It is apparent that the same amount of solids removal is achieved at all aeration rates by comparing amounts of removal for all rates of aeration at 1200 sec. Advantage of this fact may be utilized when total clarification is desired; since the clarification is essentially independent of aeration rate for sufficiently great aeration times, the amount of air expended to achieve the same degree of clarification can be minimized. The minimizing of air expenditure would be reflected in decreased costs of clarification.

From the data of Table 11 a second phenomenon may be observed. With respect to air, a maximum rate of clarification is achieved after approximately twenty seconds of aeration. Further, the maximum occurs at an aeration rate of 40 cc/min, the lowest aeration rate. Where partial clarification is desired, the process may be optimized by retaining volumes of sewage in a continuous clarification column for twenty seconds with an aeration rate of 40 cc/min. These data are presented in Fig 7. Here, the weight of solids removed per cc of air is plotted as a function of time; the maximum at twenty seconds is readily apparent. It can be seen that increasing the aeration rate resulted in a decrease in the rate of solids removal. Further, the rate of clarification with respect to air expended decreases rapidly after the first twenty seconds until 900 seconds when a constant clarification rate is achieved.

Effect of Clarification on Coliform Organism and Total Bacteria Counts

From the standpoint of sewage treatment, removal of bacteria from the lagooned sewage effluent would be an important bonus. The distribution of coliform organisms and total bacteria in the feed suspension, in the feed after pH adjustments, in the harvest, and in the clarified effluent, was investigated.

TABLE 11

EFFECT OF AERATION RATE AND PERIOD OF CLARIFICATION OF LAGOONED SEWAGE BY FROTH FLOTATION

(Sewage Adjusted to pH 3.0)

Aeration Period (sec)	Aeration Rate (cc/min)	Volume Air (sec)	Suspended Solids (mg/l)		Solids Removed	
			Feed	Effluent	mg/l	mg/cc Air
52	40	34.6	Trial 1	76	27	0.780
90	40	60.0		69	34	0.566
180	40	120.0		52	51	0.425
22	90	33.1		68	35	1.06
90	90	135.0	103	58	45	0.334
180	90	270.0		50	53	0.196
17	155	43.9		80	23	0.525
90	155	232.5		60	43	0.183
180	155	465.0		47	56	0.120
20	90	30.0	Trial 2	55	59	1.97
40	90	60.0	114	54	60	1.00
180	90	270.0		52	62	0.230
1200	90	1800.0		30	84	0.047
20	90	30.0	Trial 3	65	31	1.03
40	90	60.0		55	41	0.683
60	90	90.0		57	39	0.434
90	90	135.0	96	53	43	0.318
180	90	270.0		50	46	0.171
360	90	540.0		42	54	0.100
900	90	1350.0		42	54	0.040
20	90	30.0	Trial 4	90	5	0.167
60	90	90.0		53	42	0.466
90	90	135.0		54	41	0.304
180	90	270.0		47	48	0.177
480	90	720.0		39	56	0.078
900	90	1350.0		41	54	0.040
1200	90	1800.0		31	64	0.036
90	40	60.0		52	43	0.715
480	40	320.0		35	60	0.188
900	40	600.0	95	38	57	0.095
1200	40	800.0		24	71	0.089
20	155	51.7		63	32	0.619
60	155	155.0		57	38	0.245
90	155	232.5		55	40	0.172
180	155	465.0		44	51	0.110
480	155	1240.0		45	50	0.040
900	155	2325.0		28	67	0.029
20	40	13.3		62	33	2.48
60	40	40.0		59	36	0.900
180	40	120.0		49	46	0.384

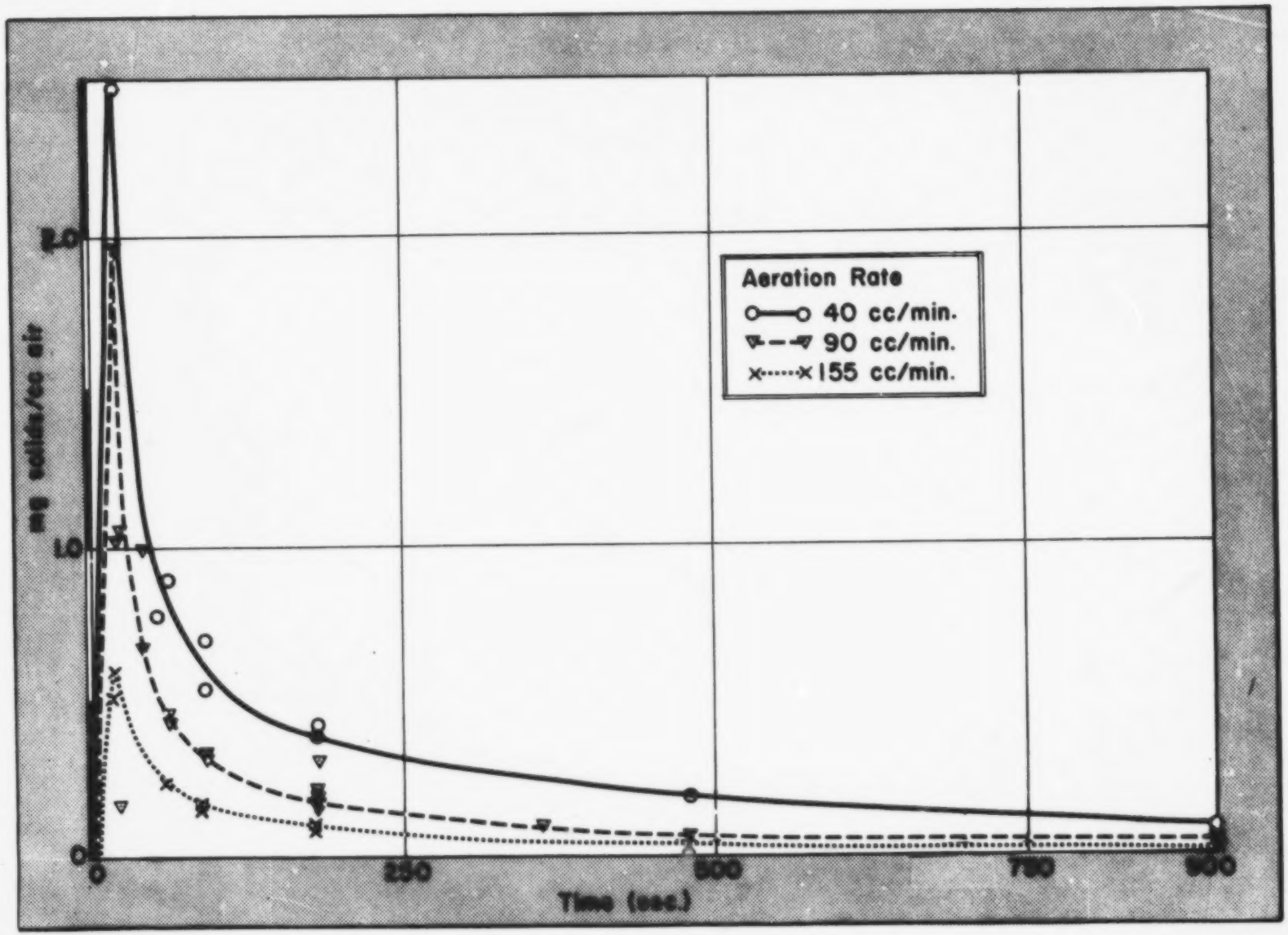

Figure No. 7 — Rate of solids removal from lagooned sewage as determined by period and rate of aeration.

The presence of members of the coliform group was determined by the membrane filter technique (8) and total bacteria determinations were made by the standard plate count method (9).

A study of the bactericidal effect of the high hydrogen ion concentration was also made.

The action of inorganic acids and alkalies in hydrolysis and coagulation of bacterial organisms by denaturing the active proteins and by coagulation or precipitating enzymes has been observed previously. "The strong mineral acids and strong alkalies have disinfectant powers proportional to their degree of disassociation in solution." (10) Both the coliform and total bacteria populations were reduced when the pH of the feed suspension was adjusted to 3.0 (Table 12).

TABLE 12

THE EFFECT OF pH 3.0 ON THE BACTERIAL POPULATION OF SEWAGE LAGOON SAMPLES

Sample One

Sample	Coliform x 10^{-3}/100 ml	Total Bacteria x 10^{-3}/100 ml	Coliform x 10^{-3}/100 ml	Total Bacteria x 10^{-3}/100 ml
Unadjusted Feed	106	28,400	16	17,700
Adjusted Feed	2	2,300	8	2,500

A reduction in total bacteria population of greater than 90% was observed upon acidification of the feed suspension; the coliform population was also greatly reduced. The acidification of the feed suspension alone is a significant step toward the production of an inocuous effluent from a sewage lagoon. However, it was found that when the acidified feed suspension was harvested, coliform organisms were completely absent from the clarified waste. Table 13 presents the results.

TABLE 13

THE EFFECT OF THE FROTH FLOTATION PROCESS ON THE DISTRIBUTION OF COLIFORM ORGANISMS AND TOTAL BACTERIA IN THE FEED SUSPENSION AND HARVEST FRACTION

Sewage Fraction	Coliform 10^{-3} x /100 ml	Total Bacteria x 10^{-3} /100 ml	Coliform 10^{-3} x /100 ml	Total Bacteria x 10^{-3} /100 ml
Adjusted Feed	2	2300	8	2500
Harvest	0	2800	2	6800
Clarified Feed	0	300	0	200

The fact that the disinfectant action of the high hydrogen ion concentration continued during the clarification process may account for the loss of viable coliform organisms in the harvest and for the relatively small increase in the total bacteria count in that fraction. Apparently, the low pH was more damaging to the coliform organisms than to the total bacterial population. These data indicate that the clarification process can produce a relatively inocuous effluent from a sewage lagoon.

Identification of Algal Flora Present in the Lagoons

The algal flora of the sewage lagoon were characterized by comparison with known flora as presented in Prescott's "Algae of the Western Great Lakes Area." (11) A microscopic examination of samples taken from the large lagoon in early August revealed a predominance of Coelastrum reticulum, followed in prominence by Scenedesmus quadricauda and Pediastrum simplex. In addition, Ankistrodesmus falcatus, Navicula sp., Scenedesmus dimorphus, Ankistrodesmus convolutus, and Arthrospira sp. were identified. After September, no one organism predominated the algal population. Benthic algae were not sampled. In the small older lagoon, the most predominant alga was Chlorella ellipsoidea, which indicated that the pond may have been working a capacity or was possibly overloaded. (12)

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